J Appl Electrochem (2012) 42:917–923
DOI 10.1007/s10800-012-0473-6

ORIGINAL PAPER

Electrochemical investigations of an anticancer drug in the presence of sodium dodecyl sulfate as an enhancing agent at carbon paste electrode

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Received: 19 April 2012 / Accepted: 18 August 2012 / Published online: 1 September 2012

Abstract In the present work, the electrochemical behavior of an anticancer drug, gemcitabine hydrochloride (GMB) was studied in the presence of a surface active agent (surfactant) at carbon paste electrode (CPE). GMB showed an oxidation peak at 1.101 V. The presence of sodium dodecyl sulfate (SDS) in the electrolyte was found to enhance the oxidation signal of GMB at CPE. The oxidation peak current of GMB in the presence of SDS was observed to be the function of accumulation time, scan rate, pH of the medium, and concentration of GMB. Accumulation time greatly influenced the peak current but did not exhibit significant influence on the peak potential. Based on this, a novel, sensitive, and convenient differential pulse voltammetric method was developed for the determination of GMB in the concentration range of 5.0×10^{-8}–3.0×10^{-4} M with a limit of detection value of 8.2×10^{-9} M. The proposed procedure was successfully applied for the determination of GMB in pharmaceutical formulations and spiked biological samples.

Keywords Gemcitabine · Carbon paste electrode · Surfactant · Enhancement effect · Differential pulse voltammetry

1 Introduction

Carbon paste electrodes (CPEs) are widely used in electrochemical investigations due to their unique characteristics such as versatility of chemical modification, renewability of the electrode surface, and compatibility with various electron mediators [1, 2]. The CPEs are cheaper and are suitable for preparing the electrode material with desired composition and pre-determined properties [3, 4]. The surfactants are known to change the electrochemical process through adsorption at interface or aggregation into supramolecular structure thereby enhancing the electrochemical signal with respect to analytes [5]. In view of this, the surfactants can be used in the development of electrochemical sensors for the determination of drugs.

GMB (2′,2′-difluoro deoxycytidine) (Fig. 1) has demonstrated to be one of the most active drugs against non-small lung cancer, pancreatic cancer, bladder cancer, and breast cancer [6–9] either as a single drug or in combination with other cytotoxic agents [10–12]. Determination of GMB and dFdU in plasma, urine, and tissues using HPLC and spectrophotometric methods were reported [13–15]. Recently, electrochemical studies and spectroscopic investigations on the interactions of GMB with DNA and their analytical applications have been reported by our research group [16]. In the present study, we have reported a simple and sensitive electrochemical method compared to the reported methods for the assay of GMB (Table 1) at CPE in the presence of sodium dodecyl sulfate (SDS). The experimental results indicated that the SDS exhibited a distinct enhancement effect on electrochemical responses of GMB at CPE. Further, the proposed method was applied for the determination of GMB in formulations and biological samples.

2 Experimental

2.1 Reagents and solution preparations

Pure sample of GMB was obtained as a gift sample from Lifecare Laboratories Pvt. Ltd., India. A stock solution of

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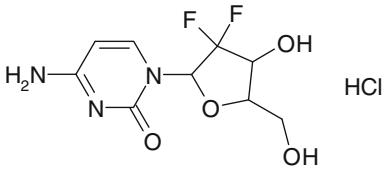

Fig. 1 Structure of gemcitabine hydrochloride

Table 1 The critical comparison of sensitivity of the proposed method with reported methods

Method	LOD ($\mu g\ ml^{-1}$)	Analytical range ($\mu g\ ml^{-1}$)	References
HPLC	0.10	0.20–10.0	[13]
Ion pair HPLC	0.07	0.35–10.0	[14]
Spectrophotometric	–	5.00–30.0	[15]
Electrochemical	0.31	1.50–224.0	[16]
Electrochemical	0.0026	0.14–89.9	Proposed sensor

GMB (5 mM) was prepared in Millipore water and stored in a refrigerator at 4 °C. Standard working solutions were prepared by appropriately diluting the stock solution with the selected supporting electrolyte. Two electrolytes viz., phosphate buffer (pH = 6–9) and Britton-Robinson (pH = 3–11) buffer were used. Surfactants, SDS (SISCO, India), cetyl trimethyl ammonium bromide (CTAB) [Sigma Aldrich, India], cetyl pyridium chloride (CPC) [Sigma Aldrich, India], and Triton X 100 (Sigma Aldrich, India) were used. Stock solutions of these surfactants (each of 5 mM) were prepared separately.

2.2 Apparatus

Electrochemical studies were carried out on a CHI-1110a Electrochemical Analyzer (CH Instruments Ltd. Co., USA, version 4.01) consisting of CPE as the working electrode, a platinum wire as the counter electrode, and Ag/AgCl as the reference electrode.

2.3 Preparation of CPE

1 g graphite powder was mixed with 210 µl paraffin oil in a mortar to form a homogeneous mixture. The mixture was pressed into the end cavity of a polytetrafluoroethylene (PTFE) cylindrical electrode body and the electrode surface was polished manually on a piece of weighing paper. The freshly prepared CPE was activated in supporting electrolyte (blank) using successive cyclic scans from 0.4 to 1.4 V until stable voltammograms were obtained.

2.4 Working procedure for voltammetric analysis

Suitable amounts of aliquots of standard GMB solutions were transferred into 10 ml volumetric flasks and completed to volume with phosphate buffer of pH 6. The solutions were then purged with pure nitrogen gas for 10 min and then cyclic/differential pulse voltammograms were recorded at CPE. The DPV conditions maintained were: pulse amplitude—50 mV; pulse width—30 ms: and scan rate—20 mV s^{-1}. All voltammograms were against the standard Ag/AgCl reference electrode.

2.5 Analysis of GMB in vials

Vials containing GMB were obtained commercially from local sources. A single vial consisted of 200 mg GMB. A portion of the powder equivalent to 5 mM GMB was transferred into a 10 ml calibrated flask and completed to volume with Millipore water. The contents of the flask were sonicated for 15 min. Appropriate solutions were prepared by suitable dilution with phosphate buffer of pH 6. The content of GMB in vials was determined by referring to the calibration graph or regression equation.

2.6 Determination of GMB in human urine and plasma samples

A spiked urine sample was obtained by treating 1.8 ml aliquot of urine with 200 µl of GMB solution (2.5 mM). A suitable aliquot of the spiked urine was then diluted with phosphate buffer without any pre-treatment to prepare appropriate sample solutions, and their differential pulse voltammograms were recorded under optimized conditions.

For the determination of GMB in plasma, spiked serum samples were prepared following the procedure reported earlier [17]. Serum samples obtained from healthy individuals (after obtaining their written consent) were stored frozen until assay. For the assay of GMB in plasma, 1 ml of GMB solution (5 mM) was added to 1 ml of untreated plasma. The mixture was vortexed for 30 s. In order to precipitate plasma proteins, the plasma samples were treated with 500 µl of 15 % $HClO_4$. The mixture was vortexed for 30 s and then centrifuged at 5,000 rpm for 5 min. An appropriate volume of supernatant liquor was transferred to a voltammetric cell containing phosphate buffer of pH 6, and voltammograms were recorded. The voltammograms of blank samples (without GMB) did not show any signal that could interfere with direct determination. The content of the drug in plasma was determined by referring to a calibration graph or regression equation.

3 Results and discussion

3.1 Electrooxidation of GMB at CPE

GMB exhibited one oxidation peak at 1.101 V in phosphate buffer of pH 6 at CPE (not shown in fig.). No peak was observed in the reverse scan suggesting that the oxidation process of GMB is irreversible. The oxidation of GMB was observed to be adsorption controlled and pH dependent at CPE.

3.2 Effect of surfactants on electrooxidation of GMB

We have shown the electrochemical behavior of GMB (Fig. 2) in the presence of different surfactants, SDS (curve a), neutral surfactant, Triton X 100 (curve b), and cationic surfactants CTAB (curve c) and CPC (curve d). The specific values of i_p and E_p in the presence of different surfactants are summarized in Table 2.

Further, the effects of concentrations of different surfactants (SDS, Triton X 100, CTAB, and CPC) on electrochemical responses of GMB were examined. For this, we have recorded cyclic voltammograms of 150 μM GMB in the presence of different concentrations of various surfactants. The oxidation responses of GMB increased with increase in the concentration of SDS, Triton X 100, CTAB, and CPC up to 800, 400, 600, and 400 μM, respectively (Fig not shown). This could be attributed to micelle effect. Beyond these concentrations of surfactants, the oxidation peak currents of GMB remained almost constant, since the aggregates of micelles inhibited the electron transfer between GMB and the electrode surface. Cyclic voltammograms of GMB in the presence (a) and the absence (c) of SDS in phosphate buffer of pH 6 are shown in Fig. 3. The peak potential of GMB was noticed to be shifted toward negative direction from 1.101 V (in the absence of SDS) to 1.090 V (in the presence of SDS) with an enhancement in peak current (~40 times). This could be attributed to hydrophobic C–H chain and hydrophilic head group of SDS that could absorb strongly at the surface of CPE via their hydrophobic interactions [18, 19]. Consequently, the adsorbed SDS induced the adsorption of GMB at the electrode surface and thus increased the surface concentration of GMB.

Table 2 Electrochemical data for the oxidation of 150 μM GMB in the presence of different surfactants

Surfactant	Concentration of surfactant (μM)	Peak potential (V)	Peak current (μA)
CTAB	600	0.965	3.295
CPC	400	0.978	1.240
Triton X 100	400	0.982	3.130
SDS	800	1.090	39.96
None	–	1.101	2.707

3.3 Influence of accumulation time

The peak current was found to increase linearly with accumulation time up to 150 s (figure not shown). Beyond 150 s, the peak current decreased. This might be due to excess adsorption of GMB at the electrode surface. So, an

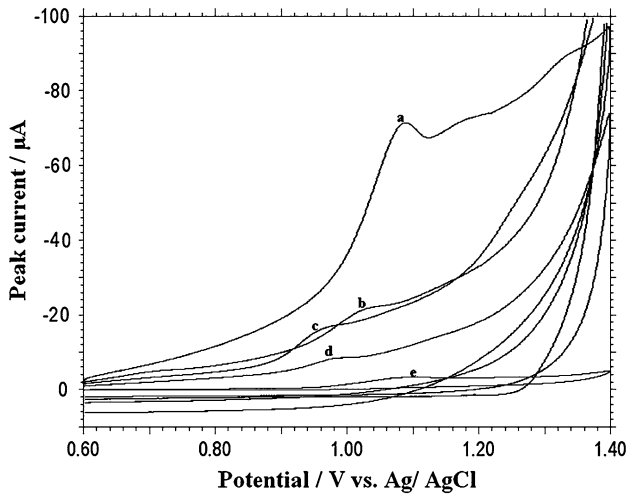

Fig. 2 Effects of different surfactants on voltammetric behavior of GMB in phosphate buffer of pH 6.0: *a* 800 μM SDS; *b* 400 μM Triton X-100; *c* 600 μM CTAB; *d* 400 μM CPC; *e* in the absence of surfactant. Scan rate: 100 mV s^{-1}; accumulation time: 150 s; GMB: 150 μM

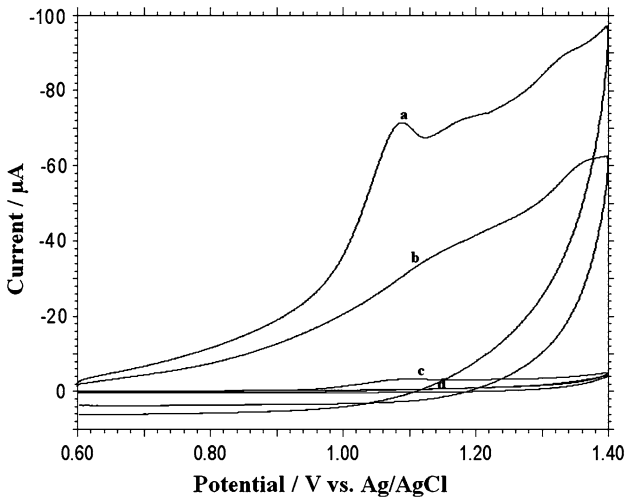

Fig. 3 Cyclic voltammograms of *a* GMB in the presence of SDS in phosphate buffer of pH 6.0; *b* SDS in phosphate buffer of pH 6.0; *c* GMB in phosphate buffer of pH 6.0, and *d* blank buffer solution (phosphate buffer of pH 6). Scan rate: 100 mV s^{-1}; accumulation time: 150 s; GMB: 150 μM; 800 μM SDS

accumulation time of 150 s was maintained for further study.

3.4 Influence of pH on electrochemical oxidation of GMB

Cyclic voltammograms of 150 μM GMB in phosphate buffer of different pH values are shown in Fig. 4. The peak potential of GMB was found to be shifted toward negative potential with increase in pH indicating that the pH of the supporting electrolyte exerted a significant influence on electrooxidation of GMB at CPE. Figure 5 shows the dependence of oxidation peak potential on pH of the electrolyte and the variation of peak current with pH of the electrolyte. The plot of peak potential *versus* pH yielded the slope of 64.9 mV pH^{-1}, which is close to the expected value of 59 mV pH^{-1}. This indicated the participation of equal number of electrons and protons in the electrode process [20]. We have carried out further investigations at pH 6 since we observed sharp and well defined peak at pH 6.

3.5 Effect of scan rate on the electrochemical oxidation of GMB

Scan rate is one of the most important experimental parameters to be investigated since the adsorbed reactant or reactant reaching the electrode via diffusion would influence the electrochemical response of GMB. By studying the effect of scan rate, we get the information with regard to electrochemical mechanism and the relationship between peak current and scan rate. Hence, we have recorded cyclic voltammograms of 150 μM GMB in the presence of SDS at different scan rates. From Fig. 6, it is evident that the oxidation peak current increased linearly with the scan rate in the range of 10–200 mV s^{-1} with positive shift in peak potential. The observed positive shift in peak potential with increase in scan rate indicated the irreversibility of GMB oxidation process. The plot of values of log i_p versus log υ in the scan rate range of 10–200 mV s^{-1} yielded a straight line with the slope of 0.85 indicating that the electrode process is adsorption controlled [21].

Positive shift in peak potential and increase in peak current with increase in scan rate was noticed in the presence of other surfactants also. The plot of values of log i_p versus log υ yielded straight lines with slope values of 0.790, 0.772, and 0.801 in scan rate ranges of 10–200,

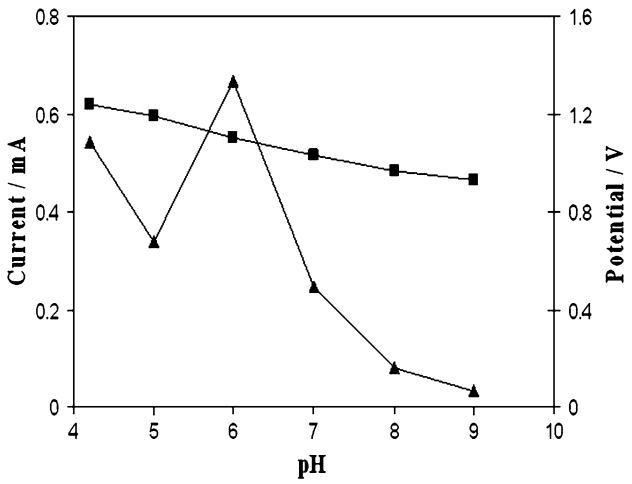

Fig. 5 Dependence of oxidation peak potential with solution pH (*square*) and relationship between peak current and solution pH (*triangle*)

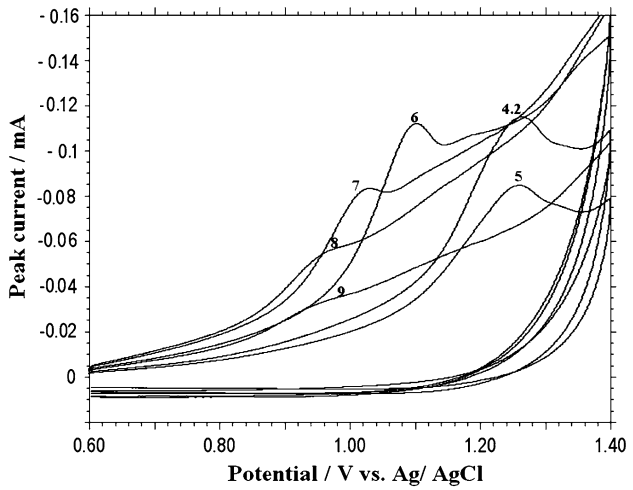

Fig. 4 Cyclic voltammograms of 150 μM GMB at different pH. Other conditions are same as in Fig. 3

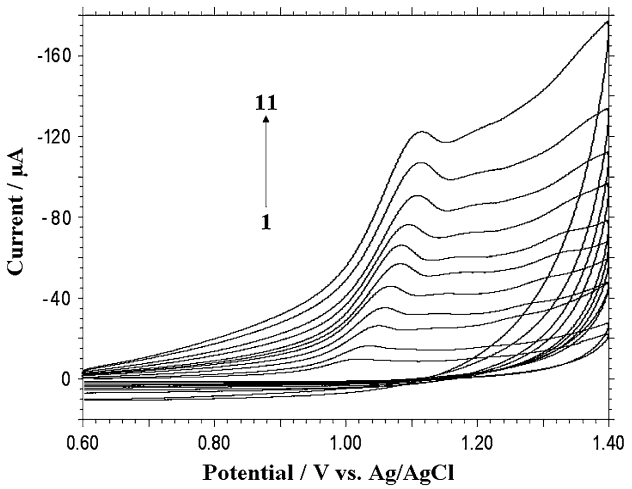

Fig. 6 Cyclic voltammograms of 150 μM GMB in presence of 800 μM SDS at different scan rates (*1–11*): 10, 20, 40, 60, 80, 100, 120, 140, 160, 180, and 200 mV s^{-1}

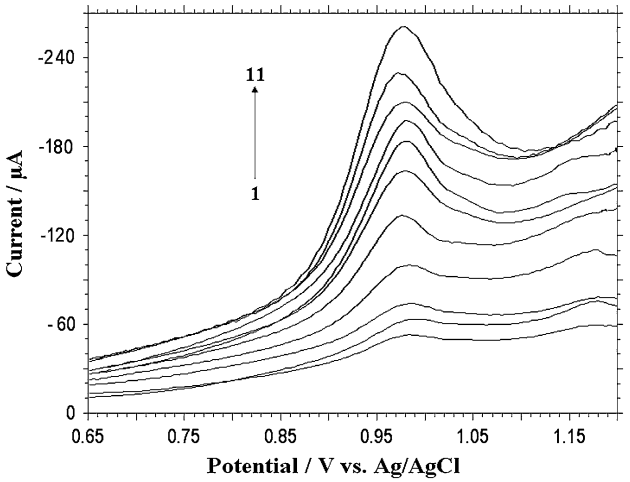

Fig. 7 Differential pulse voltammograms of GMB in phosphate buffer of pH 6 at CPE in the presence of SDS. Concentrations of GMB maintained are (*1*) 0.05, (*2*) 10, (*3*) 25, (*4*) 50, (*5*) 75, (*6*) 100, (*7*) 125, (*8*) 150, (*9*) 175, (*10*) 200, (*11*) 300 µM

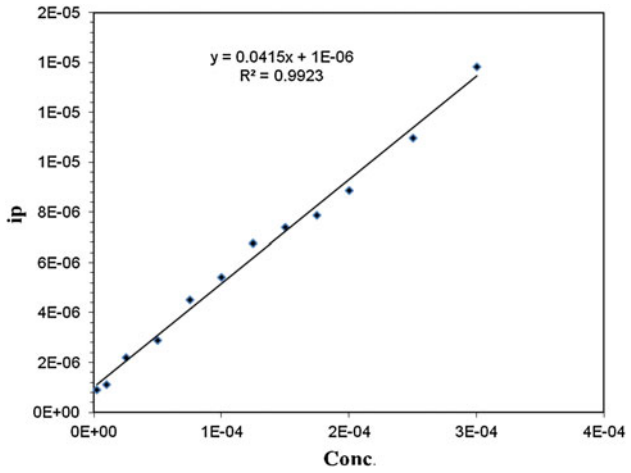

Fig. 8 The relationships between peak current and concentration of GMB. Accumulation time: 150 min, scan rate: 20 mV s^{-1}, and SDS: 800 µM

10–350, and 20–350 mV s^{-1} for Triton X 100, CTAB, and CPC, respectively, indicating that the electrode reactions are rather adsorption controlled than diffusion controlled.

4 Analytical applications

4.1 Validation of the analytical procedure

A differential pulse voltammetric method was developed for the determination of GMB in pure samples. For this, the variation of peak current (i_{pa}) with respect to concentration of GMB was investigated. The differential pulse voltammograms of GMB at different concentrations are shown in Fig. 7. Under the optimized experimental conditions (pulse amplitude, 50 mV; pulse width, 30 ms; scan rate, 20 mV s^{-1}), linear relationship between the peak current and concentration of GMB was noticed in the range of 5×10^{-8}–3×10^{-4} M (Fig. 8). In this concentration range, the response was noticed to be adsorption controlled. The analytical characteristics of the calibration plot are summarized in Table 3. Validation of the optimized procedure for quantitative assay of GMB was examined by the evaluation of limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, and recovery studies. The values of LOD and the LOQ were calculated using the equations shown below [22, 23]:

$$\mathrm{LOD} = 3\,s/m; \mathrm{LOQ} = 10\,s/m$$

where s is the standard deviation of the intercept ($n = 5$) and m is the slope of the calibration curve.

The values of LOD and LOQ were calculated to be 8.95 and 29.8 nM, respectively. Low values of both LOD and LOQ confirmed the sensitivity of the proposed method. The RSD values were calculated for inter-day assay by analyzing 10, 50, and 100 µM GMB ($n = 6$). The corresponding RSD values (Table 3) were calculated to be 2.09, 2.53, and 1.94 %, respectively. The RSD values (Table 3) for intra-day assay of 10, 50, and 100 µM GMB solutions ($n = 6$) were found to be 2.33, 2.21, and 2.43 % indicating good repeatability of the results. The results of the intra- and inter-day assay were subjected to F test to compare intra- and inter-day precision of the proposed method. The calculated values (F_{cal}) were found to be less than F_{crit} values at 95 % confidence level. The corresponding results are shown in Table 4. This confirmed that there was no significant difference between two sets of data at 95 % confidence limit and hence the method is precise.

Table 3 Characteristics of calibration plot of GMB

	DPV
Linearity range (µM)	0.05–300
LOD (nM)	8.95
LOQ (nM)	29.8
Inter-day assay RSD[a] (%)	2.53
Intra-day assay RSD[a] (%)	2.43

[a] Average of six determinations

4.2 Determination of GMB in pharmaceutical dosages

The suitability of the proposed electrochemical method was demonstrated by analyzing GMB in its vials and the results were found to be satisfactory (Table 5).

Table 4 F test results of intra- and inter-day assay of GMB

GMB (μM)	*n*	F_{cal}	F_{crit}
Intra-day assay			
10	6	2.12	5.05
50	6	3.08	5.05
100	6	0.06	0.19
Inter-day assay			
10	6	2.24	5.05
50	6	1.37	5.05
100	6	0.013	0.19

Table 5 Determination of GMB in pharmaceutical formulation

Vial	Labeled amount (mg)	Found (mg)	Recovery (%)	RSD[c] (%)
Heterogem[a]	200	198.7	99.35	1.95
Cytogem[b]	200	199.8	99.90	1.80

[a] Marketed by Hetero. Ltd., India

[b] Marketed by Dr. Reddy's laboratories Ltd., India

[c] Average of six determinations

Table 6 Results of analysis of GMB in spiked human urine and serum sample

GMB added (μM)	*n*	Amount found (μM)	Average recovery (%)	RSD (%)
Urine samples				
10	5	9.80	98.02	2.04
50	5	49.43	98.87	2.50
100	5	99.70	99.70	1.93
Serum samples				
10	5	9.90	99.06	2.06
50	5	49.51	99.02	2.49
100	5	99.18	99.18	2.23

4.3 Determination of GMB in urine and blood samples

The proposed method was also applied for the determination of GMB in spiked urine samples of healthy volunteers. The recovery of GMB from urine samples was determined by spiking the drug-free urine with known amounts of GMB, and differential pulse voltammograms were then recorded. The amounts of GMB in urine samples were then evaluated from the calibration graph. The results of the analysis are listed in Table 6. Higher recovery values and and lower RSD values indicated high accuracy and precision of the proposed method.

Further, the proposed method was also applied to the assay of GMB in spiked human serum samples of healthy volunteers. For this experiment, drug-free serum samples were spiked with 10, 50, or 100 μM of GMB; differential pulse voltammograms were then recorded. The results of the analysis are summarized in Table 6 and noticed to be satisfactory.

5 Conclusions

Enhanced electrochemical responses of GMB were noticed in the presence of SDS. A simple and sensitive differential pulse voltammetric method was developed. This method could be readily adopted for quantitative assay of GMB in pharmaceutical formulations and in biological samples.

Acknowledgments One of the authors (Nagappa L. Teradal) acknowledges DST, New Delhi for the awarding INSPIRE Fellowship (IF110339). The authors are also grateful to the University Grants Commission, New Delhi, India, for financial assistance (F. no. 34/374/2008 (SR) dated 30-12-2008).

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